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Kidney International, Vol. 31 (1987), pp. 85–92

CLINICAL INVESTIGATION

Renal excretion and cyst accumulation of $\beta_2$microglobulin in polycystic kidney disease

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Renal excretion and cyst accumulation of $\beta_2$microglobulin in polycystic kidney disease. To determine the extent to which proximal tubule function is altered $\beta_2$microglobulin (β_2m), creatinine and Na were measured in serum, urine and cyst fluid of patients with autosomal dominant polycystic kidney disease and various degrees of renal insufficiency. Fractional excretion (FE_{β_2m}) was 0.11 ± 0.03% in six normal subjects and 0.13 ± 0.05% in nine patients with serum creatinine levels less than 1.6 mg/dl. In five patients with serum creatinine levels above 3.0 mg/dl, FE_{β_2m} was elevated (range 3.5 to 196%) and serum levels were higher than normal (30,600 ± 6,910 μg/liter vs. 1,268 ± 111). In seven patients β_2m levels in 33 proximal cysts (cyst/serum Na > 0.8) equalled those in serum (cyst/serum β_2m 0.98 ± 0.20), whereas in 21 distal cysts (cyst/serum Na < 0.4) β_2m was less than in serum (cyst/serum β_2m 0.17 ± 0.07). Analysis of fluid in two patients with polycystic kidney nephrectomy several weeks post-transplant indicated that proximal cyst epithelium is permeable to β_2m, but less so than to creatinine or urea. These studies show that proximal cysts cannot develop or maintain gradients for β_2m, whereas distal cysts maintain low levels of the protein despite end–stage renal failure. The normal FE_{β_2m} values in nonazotemic autosomal dominant polycystic kidney disease patients and the low distal cyst levels of β_2m in end–stage kidneys indicate that the cystic proximal nephrons do not contribute appreciably to the final urine. These findings are consistent with the view that a relatively small number of cystic tubules ultimately compromise the function of non-cystic nephrons.

Autosomal dominant polycystic kidney disease (ADPKD) is a common heritable renal disorder in which the kidneys progressively enlarge as normal parenchyma is replaced by innumerable fluid–filled cysts [1–3]. Microdissection of acid–hydrolyzed nephron segments and analysis of the chemical composition of cyst fluid from kidneys of patients with ADPKD indicate that the cysts are derived from and may remain connected to renal tubules [4–6]. The cyst epithelium appears to function, even in "end stage" kidneys, in several ways similar to the nephron segments from which the cysts derive. The intercellular junctions are permeable to lanthanum, and transepithelial gradients of Na, K and osmolality are not effectively maintained by "proximal" cysts [7]. By contrast, "distal" cysts are lined by cells with tight junctions that restrict the movement of the lanthanum tracer; this allows for the long–term maintenance of relatively steep transepithelial gradients of creatinine, hydrogen ion, sodium and potassium [7–9]. Recent direct studies of cyst walls in vitro have confirmed the ability of certain cysts to actively transport solutes [10].

Received for publication March 31, 1986
and in revised form July 7, 1986

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$\beta_2$microglobulin (β_2m) is a normal plasma protein that is produced by a variety of cells in the body, filtered by glomeruli and extensively reabsorbed by the proximal tubules. In the course of reabsorption the proximal tubule cells metabolize β_2m to the constituent amino acids that are returned, in part, to the plasma [11]. The fractional excretion of β_2m in normal subjects is less than 1% of the filtered load [11, 12]. Thus, the plasma level of β_2m is primarily dependent on the rate of production and glomerular filtration, whereas the urinary excretion rate depends on the rate of β_2m filtration by glomeruli and the extent to which the protein is absorbed and metabolized by proximal tubule cells.

β_2m is present in serum, urine, CSF, saliva and other biological fluids primarily as a free monomer [13–15]. Because of its size (11,600 Daltons, Stoke's radius 1.6 nm) it is reasonable to expect that this solute is filtered by glomeruli of polycystic kidneys and that the concentration of β_2m in renal cysts reflects the capacity of the afferent tubular epithelium and the cyst epithelium to transport and metabolize β_2m. In the present study we determined β_2m levels in cyst fluid, serum and urine of patients with ADPKD to evaluate the utility of this unique protein as an additional marker of renal tubule and cyst function.

Methods

We studied two distinct sets of patients with ADPKD. Fourteen ambulatory patients were followed in the ADPKD clinic at the University of Kansas Medical Center; eight other patients and one ambulatory patient (D.S.) had one or both polycystic kidneys surgically removed. Consent was obtained for the collection of serum, cyst fluid and urine specimens.

Renal excretion of β_2m

Blood and mid-stream urine samples were collected between 8 a.m. and 1 p.m. from 14 ambulatory patients with ADPKD (seven females and seven males) and six normal persons (three females and three males). The normal subjects had no detectable renal disease and no family history of renal disease. Serum and urine specimens from each subject were collected concurrently and refrigerated immediately. Twenty-four hour urine samples were not consistently available. Blood samples were centrifuged the day of collection. Serum and urine samples were tightly sealed and stored at −20°C.

Distribution of β_2m in kidney cysts

We obtained clear fluid from superficial (subcapsular) cysts and serum from nine patients (five females and four males) with ADPKD. Eight of these patients are described and detailed methods of sample collection are outlined in Huseman et al [9]. Patient D.S. was added to the previous group and samples were processed identically. All sealed specimens were stored at −20°C in tightly–capped glass vials. One patient was non-azotemic (T.H.), the kidneys having been donated ostensibly for cadaveric renal transplantation. Two patients had received kidney transplants and had their cystic kidneys removed six (A.B.) and 12 weeks (M.G.) post-transplant because of intractable renal infection. Both of these patients had serum creatinine levels below 2.0 mg/dl within one week post-transplant. Serum samples were obtained prior to and following cadaveric renal transplantation. Patient A.B's serum, drawn at the time of nephrectomy, was not available for β_2m assay. As the patient has a normally functioning renal allograft, a recent serum sample was used for β_2m determination. The remaining five patients were treated with thrice–weekly chronic hemodialysis using hollow–fiber or parallel plate dialyzers. In these patients, nephrectomies were performed for intractable infection, pain or in preparation for renal transplantation.

Analysis of data and calculations

Sodium and creatinine in serum, cyst fluid and urine were measured in the University of Kansas Clinical Pathology Laboratories by an Autoanalyzer technique. By convention "proximal" cysts have cyst/serum Na ratios > 0.8, and "distal" cysts have ratios < 0.4 [9]. β_2m was determined in serum, cyst fluid and urine with the Phadezym $\beta_2$micro Test kit, a competitive immunoassay method obtained from Pharmacia Diagnostics of Sweden. In this assay, a known amount of enzyme–labelled β_2m competes with β_2m in each sample for binding sites on a Sephadex anti-β_2m complex. The enzyme, Sephadex and an aliquot of serum, urine or cyst fluid were incubated together for one hour at 37°C. Each sample and five different concentrations of standard were run in duplicate. Bound and free β_2m were separated by centrifugation. A reducing agent and substrate supplied by the manufacturer were added to the solution to release and then hydrolyze the enzyme during a one hour incubation at 37°C. The product was yellow with an absorbance maximum of 420 nm. Hydrolysis was terminated by addition of the manufacturer's Stop solution.

Absorbance levels measured at 420 nm with a Gilford Stasar III spectrophotometer were inversely proportional to the concentration of β_2m in the sample. A linear standard curve ranging from 10 to 500 μg/liter β_2m concentration was constructed for each assay. Results of all unknowns were determined by interpolation between standards and expressed in μg/liter after correction for the dilution factor. β_2m levels were considered undetectable if absorbance readings were higher than 0.830 in undiluted samples; the lower limit of detection was 10 μg/liter. Serum, cyst fluid and urine were generally diluted 1:50 to 1:200, 1:5 to 1:200, and 1:5 to 1:200 with buffer, respectively. β_2m recovery in normal serum and urine was 72 and 77%, respectively.

To evaluate reproducibility of the assay, we collected 10 mls of serum from a normal subject and separated it into 200 μl aliquots that were frozen. An aliquot was run in duplicate along with each assay to assess reproducibility from day to day. Each serum aliquot was used for one assay and then discarded. In 14 separate assays covering four months, the mean absorbance of this serum (± SD) was 0.644 ± 0.094. The calculated β_2m concentration of the serum (mean ± SD) was 1610 ± 568 μg/liter. The lower coefficient of variation of the absorbance values (14.6%) compared to the final β_2m concentrations (35.3%) probably reflects variation among the different β_2m standards supplied with the individual assay kits. To minimize the impact of this variability for the critical comparisons between proximal and distal cyst fluids, serum and urine samples for a given subject were run on the same day. Consequently, intra-subject comparisons had less inherent methodologic variability than inter-subject comparisons.

Five separate collections of serum from an ADPKD patient spanning a 2-1/2 year period of hemodialysis were assayed concurrently. The measurements were repeated one month later to determine the effect of storage. The mean β_2m concentration of the five separate serum samples was 31,300 ± 3230 μg/liter (SD). The mean ratio of individual samples assayed one month later was not significantly different from 1.0 (0.954 ± 0.028, SE). The β_2m concentrations for one of this patient's proximal cysts assayed in duplicate one month apart was 29,600 and 32,500 μg/liter, respectively. These measurements indicate that serum β_2m levels are relatively constant in dialysis patients, and that serum and cyst fluid can be stored frozen without an appreciable change in β_2m levels.

Other workers have reported that β_2m activity is decreased in strongly acid urine [14, 16]. We obtained urine from a normal subject and added HCl or NaOH to separate 2 ml aliquots of the urine to give four different samples with pH ranging from 4.3 to 7.5. β_2m levels were assayed in duplicate on the same day the samples were prepared. β_2m concentrations of 1120, 725, 1090, and 1040 μg/liter for pH values of 4.3, 5.3, 6.2, and 7.5, respectively, revealed no consistent effect of urine pH on the β_2m levels.

To determine the effect of pH on β_2m levels in cyst fluid, we utilized one of the samples with the highest β_2m levels. To a 2 ml aliquot of this fluid sufficient isotonic HCl was added to reduce the pH to 5.0. An equal volume of isotonic Ringer's bicarbonate solution was added to a second 2 ml aliquot of the distal cyst fluid. Both samples were frozen immediately and retrieved three weeks later. β_2m was measured in triplicate and pH was estimated by indicator paper. The mean β_2m levels of 19,800 ± 301 and 22,200 ± 1490 μg/liter (SE) for pH 5.0 and 8.0 were not statistically different. These studies indicate that β_2m levels are stable in frozen urine and cyst fluid and relatively insensitive to pH values between 4.3 and 8.0.

Since cyst fluids may contain a variety of plasma proteins [4, 8], we used high performance liquid chromatography (HPLC) and proximal cyst fluid to separate the macromolecules [17]. HPLC was performed with a VISTA series 5500 (Varion Co., Walnut Creek, California, USA) using a TSKG 3000 SW column. The elution buffer was 0.05 M Na_2PO_4 at pH 7.0 in 0.15 M NaCL. Absorbance was measured at 230 nm. Undiluted cyst fluid (40 μl) was injected into the column and effluent aliquots were collected at one and one–half minute intervals. β_2m levels and absorbance were determined in each fraction (Fig. 1). Most of the proteins in the cyst fluid had molecular weights > 12,000

Table 1. Renal excretion of β_2m

Subject	Sex	Age years	Creatinine serum *mg/dl*	β_2m serum *μg/liter*	β_2m urine *μg/g creat.*	FE_{β_2m}
Normal						
1	F	30	0.6	1130	90	0.00048
2	F	38	0.7	1180	247	0.00147
3	M	28	0.7	1120	131	0.00082
4	M	48	0.9	1150	273	0.00213
5	M	38	0.9	1210	155	0.00012
6	F	29	1.0	1820	279	0.00153
Mean ± SE			0.8 ± .06	1268 ± 111	196 ± 33	0.00109 ± 0.00031
Non-azotemic ADPKD						
WD	F	55	0.8	1350	243	0.00144
SW	F	37	0.9	1190	99	0.00075
AA	F	29	0.9	2510	55	0.00020
SO	F	56	0.9	3225	191	0.00055
CR	M	39	1.1	4250	275	0.00071
PN	M	50	1.1	3080	234	0.00083
TW	M	35	1.3	5850	2411	0.00535
DC	M	40	1.6	3840	196	0.00082
MV	F	61	1.6	5950	230	0.00062
Mean ± SE			1.1 ± 0.1	3472 ± 570	437 ± 248	0.00125 ± 0.00052
Azotemic ADPKD						
ME	F	52	3.9	12700	55980	0.17200
YS	M	48	5.8	17800	10770	0.03500
DS[a]	M	48	14.0	31900	65800	0.28900
NC[a]	M	52	14.0	49600	190900	0.53800
MA[a]	F	46	15.0	41000	535000	1.96000
Mean ± SE			10.5 ± 2.3	30600 ± 6910	171700 ± 95600	0.5988 ± 0.3500

[a] Hemodialysis 3 x week; M, Male; F, Female

daltons. The peak concentration of β_2m coincided with a minor protein fraction in the 12,000 molecular weight range, indicating that the immuno-assay could discriminate between β_2m and other proteins in cyst fluid.

Apparent fractional delivery of β_2m (FD) was calculated for each cyst from β_2m and creatinine concentrations using the equation:

$$\text{Equation 1: } FD_{\beta_2m} = \frac{\text{cyst } \beta_2\text{m/serum } \beta_2\text{m}}{\text{cyst creatinine/serum creatinine}}$$

Fractional excretion of β_2m in the urine (FE_{β_2m}) was calculated using the equation:

$$\text{Equation 2: } FE_{\beta_2m} = \frac{\text{urine } \beta_2\text{m/serum } \beta_2\text{m}}{\text{urine creatinine/serum creatinine}}$$

Results

Renal handling of β_2m

In six normal adults (three female, three males) FE_{β_2m} averaged 0.0011 ± 0.0003 with a mean β_2m serum level of 1268 ± 111 μg/liter (Table 1, Figs. 2, 3). The highest serum creatinine level in the normal group was 1.0 mg/dl. In nine (five females, four males) non-azotemic ADPKD patients FE_{β_2m} averaged 0.00125 ± 0.00052, which is not significantly different from the normal subjects (Table 1, Fig. 3). The serum creatinine

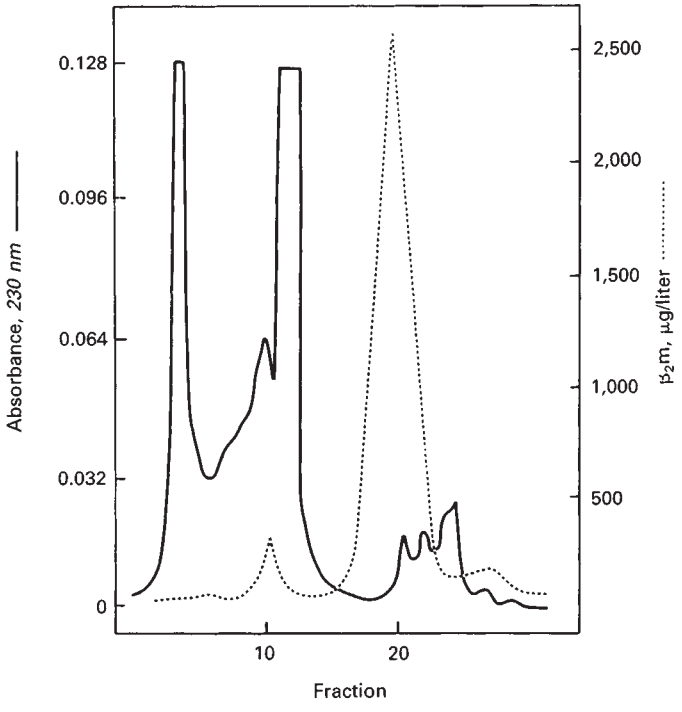

Fig. 1. *HPLC analysis of proximal cyst fluid molecular weight.* 40 μl of cyst fluid was injected and fractions collected every 1.5 minutes. Dashed line shows β_2m concentration and solid line light absorbance due to protein. Molecular weight decreases from left to right. The major β_2m peak coincides with 12,000 Daltons. Major absorbance peaks to the left represent globulins and albumin.

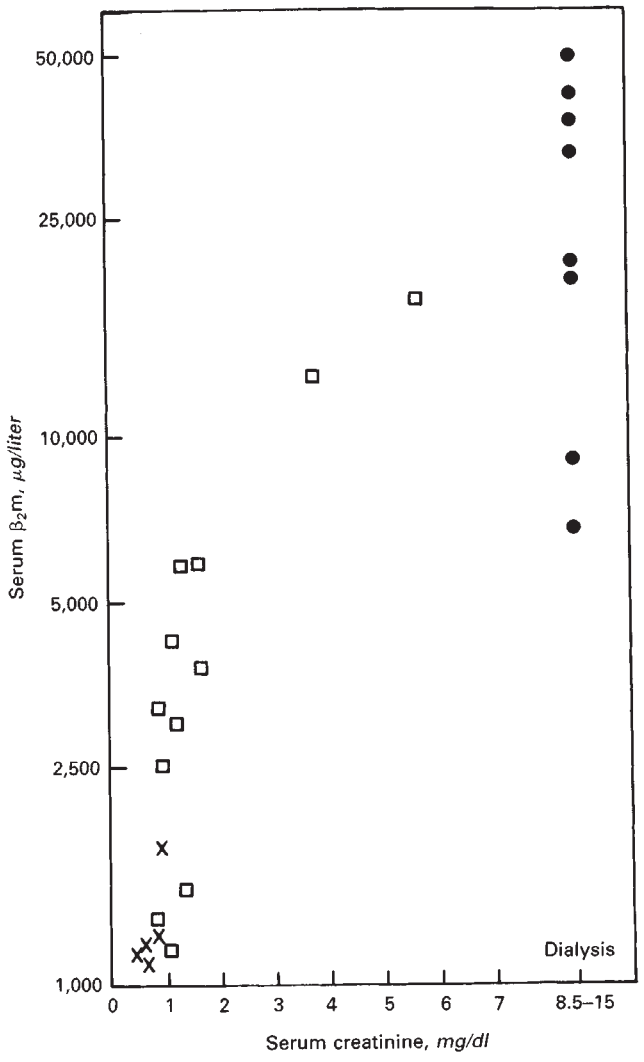

Fig. 2. *Relation between serum creatinine and β_2m levels in normal and ADPKD subjects.* Dialysis patients had creatinine values between 8.5 and 15 mg/dl. Symbols are: (□) non-dialysis PKD; (●) dialysis PKD; and (×) normal.

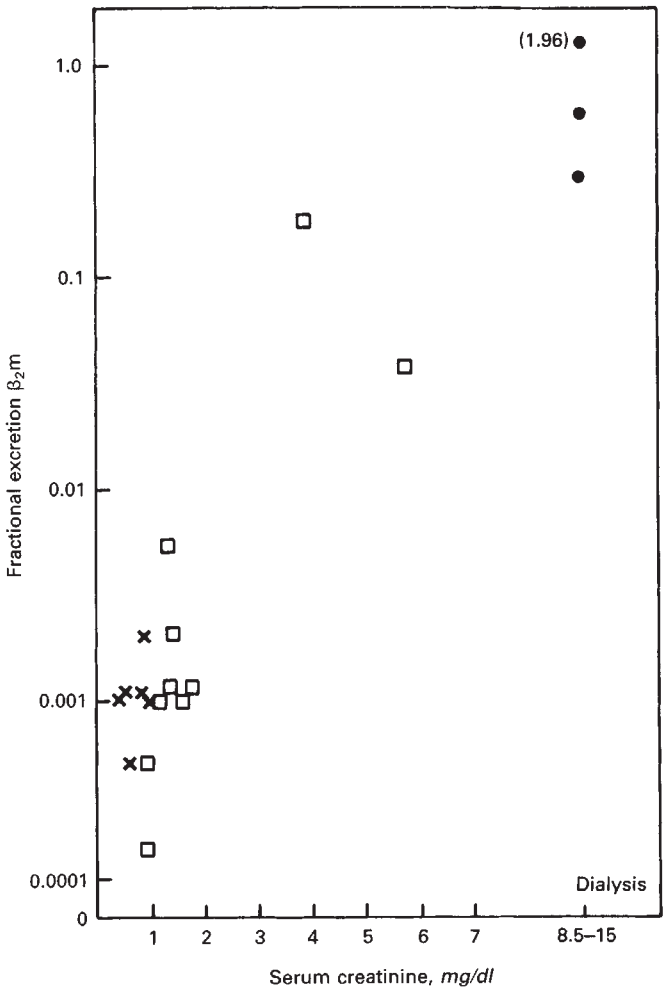

Fig. 3. *Relation between serum creatinine level and FE_{β_2m} in normal and ADPKD subjects.* Same subjects as Figure 2 except for four dialysis patients from whom urine samples could not be obtained. Symbols are the same as Figure 2.

levels exceeded those in the normals, the highest being 1.6 mg/dl. The serum β_2m levels (mean ± SE) of the non-azotemic patients were also higher than the normals, 3472 ± 570 μg/liter, $P < 0.05$. The rate of urinary β_2m excretion, judged from the urinary β_2m/creatinine ratio, was not different between the normal subjects and the non-azotemic ADPKD patients (Table 1).

In contrast to the non-azotemic ADPKD patients, those with moderate to severe decreases in GFR, as judged by serum creatinine levels exceeding 3.0 mg/dl, showed clear abnormalities in β_2m excretion (Table 1, Fig. 2). In two azotemic non-dialysis patients the serum levels of β_2m exceeded 10,000 μg/liter, FE_{β_2m} was greater than 0.035 and the urinary excretion rate of β_2m was over 10,000 μg/g creatinine. In the azotemic hemodialysis patients the serum β_2m levels exceeded 30,000 μg/liter, FE_{β_2m} was over 0.289 and the urinary excretion rate exceeded 65,000 μg/g creatinine.

Cyst levels of β_2m

We measured β_2m levels in fluids aspirated from 33 proximal and 21 distal cysts in seven patients with ADPKD; one was non-azotemic (T.H.) and six were regularly hemodialyzed (Table 2). The relative numbers of proximal and distal cyst fluid samples do not reflect the proportion of these types of cysts in ADPKD subjects [9]; rather the samples reflect the availability of adequate volumes of fluid in each subject. With the exception of D.S., all ADPKD patients were included in an earlier report [9].

The average cyst/serum ratio of β_2m in proximal cysts from six dialysis patients and T.H. was 0.985 ± 0.202, which is not different from 1.0 (Table 2). The range of ratios was wide, however, extending from 0.409 to 1.754. By contrast, the mean cyst/serum β_2m ratio in distal cysts was 0.171 ± 0.068 which is statistically lower ($P < 0.01$) than the proximal ratios. In distal cysts the ratios ranged from 0 to 0.467. Of the 21 distal cysts, 11 had no detectable β_2m. By contrast, β_2m was not detected in only one of 33 proximal cysts. In T.H., the non-azotemic

Table 2. Cyst concentrations of β_2M and creatinine[a]

Patient	Sex	Age years	Serum		Proximal cysts			Distal cysts		
			Creatinine *mg/dl*	β_2m *μg/liter*	Creatinine *mg/dl*	β_2m *μg/liter*	FD_{β_2m}	Creatinine *mg/dl*	β_2m *μg/liter*	FD_{β_2m}
Dialysis										
PB	F	43	8.9	8,900	8.0	4,840	0.60	43.5	8,520	0.189
					8.0	24,500	1.55	29.0	69	0.002
					7.0	20,600	1.40			
					8.0	19,600	1.24			
					7.0	8,500	0.62			
RC	F	64	11.1	36,800	7.5	18,000	0.72	39.0	0	0
					9.0	16,100	0.54	64.0	0	0
					7.5	19,700	0.79			
					8.5	21,500	0.76			
					8.5	0	0			
MW	F	47	11.5	20,200	10.0	24,500	1.39	6.0	57	0.005
					9.5	18,100	1.08	106.0	54	0.003
					9.5	28,600	1.70	38.0	0	0
					9.5	32,200	1.91			
					10.0	37,600	2.14			
					11.0	33,300	1.72			
DS	M	48	12.0	42,500	9.6	19,100	0.56	35.0	11,200	0.090
					14.8	30,000	0.57			
					14.8	30,000	0.41			
TB	M	46	12.8	19,400	10.0	23,400	1.55	86.0	16,700	0.128
					10.0	4,350	0.29	106.0	54	0.0003
					11.0	12,700	0.76	38.0	0	0
					10.0	12,000	0.79			
					10.0	4,000	0.26			
JS	M	48	15.3	6,550	13.9	9,450	1.58	8.0	0	0
					13.0	9,750	1.75	15.5	0	0
					13.9	17,500	2.93	13.9	0	0
					116.0	105	0.002	9.5	43	0.011
								23.0	0	0
								67.0	0	0
								24.5	0	0
								21.6	0	0
								91.9	0	0
Kidney Donor										
TH	F	34	1.13	1,510	0.7	1,170	1.44	7.4	268	0.031
					0.7	1,570	1.93			
					0.5	1,110	1.91			
					0.7	970	1.19			
					1.0	875	0.75			
Post-transplant										
AB	M	36	1.4	3,830	1.4	9,300[b]		41.0	540[b]	
					1.5	8,300		93.9	297	
					1.4	8,800		105.0	297	
					1.3	6,500		101.0	32	
					1.2	8,500		13.0	0	
MG	F	43	1,7	5,400	1.5	8,250[b]				
					1.5	8,550				
					2.0	9,450				
					1.5	15,000				
					1.5	11,000				

[a] Zero values reflect β_2m levels below the limits of detection
[b] FD values not calculated since cyst concentration not at steady state

kidney donor, only one distal cyst fluid sample was available for assay and the β_2m level was lower than in any of the five proximal cysts assayed from this kidney.

In two patients (A.B. and M.G.) the cyst fluid samples were obtained after restoration of normal serum creatinine and urea levels following renal transplantation. In M.G. no distal cysts were available for assay. In A.B. the β_2m levels in the proximal cysts were higher than in the distal cysts (Table 2).

The mean β_2m values for all subjects in this study were uniformly lower in distal in comparison to proximal cysts (Fig. 4A). Moreover, the apparent fractional delivery of β_2m (FD_{β_2m}), utilizing creatinine as the reference marker of glomerular filtra-

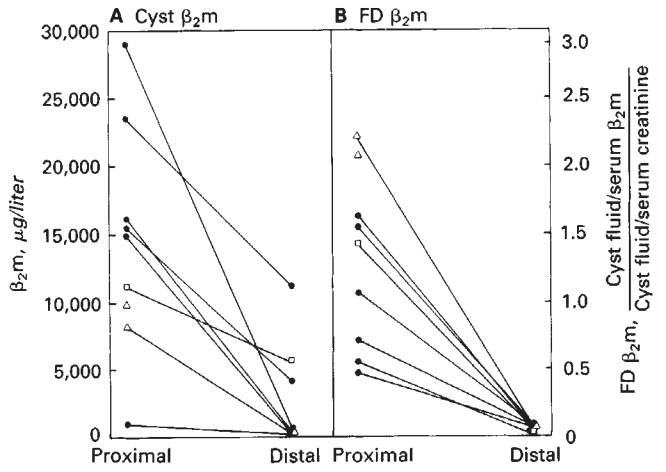

Fig. 4. *Cyst fluid β_2m levels.* **A.** Mean β_2m levels in dialysis and non-dialysis patients. One of the non-dialysis patients was never azotemic and two of them were not azotemic following renal transplantation. Lines connect mean "proximal" and "distal" β_2m values in each subject. One patient had no distal samples. **B.** Mean fractional delivery values for β_2m (FD_{β_2m}) in same subjects shown in A. Symbols are: (●) dialysis; and non-dialysis (□) non-azotemic and (△) post-transplant.

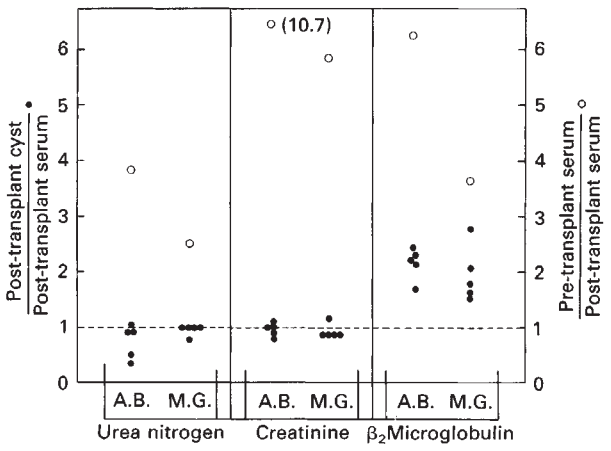

Fig. 5. *Equilibration of urea nitrogen, creatinine and β_2m in proximal cysts.* Initials refer to patients. Open circles (○) show values for pre-transplant serum/post-transplant serum for each solute, an estimate of the relative concentrations of the substances in proximal cyst fluid prior to renal transplantation. Solid circles (●) show values for post-transplant cyst fluid/post-transplant serum for each solute. The dashed horizontal line defines equilibration between cyst fluid and serum. Values of creatinine and urea nitrogen were not different from unity for both patients. Values for β_2m in post-transplant cyst fluids were significantly different from unity (Δ 1.14 ± 0.13 and 0.92 ± 0.22, $P < 0.01$), and also differed from the estimated pre-transplant cyst fluid levels (Δ 4.11 ± 0.13 and 1.67 ± 0.23, $P < 0.01$).

tion, was also uniformly lower than in each of the proximal cysts (Fig. 4B). As will be discussed later, creatinine is not an ideal filtration marker as it penetrates proximal cyst epithelium. Distal cysts, however, are sparingly permeable to creatinine and to β_2m, and the FD_{β_2m} calculation serves to estimate the degree to which β_2m levels are reduced in distal cyst fluid in comparison to proximal cyst fluid and to serum. In the six hemodialysis patients, the mean FD_{β_2m} in the distal cysts was 0.039 ± 0.018, with a range of 0 to 0.095.

Cyst β_2m levels in post-transplant patients

The availability of polycystic kidneys from two ADPKD subjects several weeks after a successful kidney transplant provided the opportunity to determine the extent to which β_2m, creatinine and urea equilibrated between cyst fluid and serum (Table 2, Fig. 5). Proximal fluid samples were available in both cases, and though we did not have a serum sample for β_2m analysis from A.B. at the time of nephrectomy (this sample was lost after the initial analysis), we obtained a sample several years later when the serum creatinine level was unchanged from the pre-nephrectomy value. Post-nephrectomy serum creatinine and urea nitrogen levels had been measured previously in A.B. Since in dialysis patients the proximal cyst fluid to serum ratios of β_2m, creatinine and urea nitrogen were virtually unity, the pre-transplant serum levels of β_2m, creatinine and urea nitrogen gave an estimate of the proximal cyst fluid levels expected in each patient prior to renal transplantation. If, after transplantation, the cyst fluid β_2m, creatinine and urea nitrogen levels completely equilibrated with serum (plasma) values, one would expect the post-transplant cyst fluid/post-transplant serum ratios to approach unity. In fact, we observed equilibration of urea nitrogen and creatinine in five proximal cysts from each subject; however, the β_2m levels were distinctly greater than one in the same proximal cyst fluids (Fig. 5). This analysis shows that the β_2m levels of proximal cysts decreased from the estimated pre-transplant values in the two patients, although they did not equilibrate completely with the serum values. The same type of analysis could not be done for β_2m permeability of distal cysts as we could not estimate the initial azotemic condition as we did for proximal cysts. It is important to note, however, that the distal cyst β_2m levels in patient A.B. (Table 2) remained below serum levels and appreciably greater than zero, consistent with low permeability of the cyst wall to β_2m.

Discussion

Previous studies indicate that cysts in ADPKD are derived from tubular segments and continue to function to a variable extent throughout the life of the patient [1, 4, 6–8, 10, 18]. The high concentrations of creatinine in distal cysts indicate that the fluid within them is derived in part from glomerular filtrate. Since β_2m is not actively secreted by proximal or distal tubules [11, 19–21], the β_2m in cysts may have been carried there in glomerular filtrate or may have diffused into the cysts from the interstitium. Proximal cysts are undoubtedly permeable to β_2m since the protein partially equilibrated across cyst walls in two patients after the extracellular fluid composition was normalized by cadaveric renal transplantation (Fig. 5). Filling of the cysts with non-azotemic glomerular filtrate does not explain the fall of the β_2m levels in the proximal cysts after successful transplantation, since one would expect the β_2m levels to equilibrate as did creatinine and urea, and this was not observed. It is also clear that the walls of the proximal cysts are only sparingly permeable to β_2m since they did not completely equilibrate with plasma, despite contact times of six and 12 weeks. Thus, the current study is consistent with the view that β_2m can diffuse relatively slowly across cyst epithelium down a concentration gradient and that the β_2m in proximal cysts

derives in part from the diffusion of interstitial β_2m into the cysts. Consequently, the protein can not be considered to be a unique marker to indicate that glomerular filtrate fills cysts of proximal origin.

By contrast, distal cyst epithelium is virtually impermeable to β_2m, since some cysts can maintain vanishingly low levels of β_2m despite serum levels exceeding 10,000 μg/liter. The source of β_2m in these distal segments may be the glomerular filtrate or fluid delivered from β_2m-rich afferent proximal cysts with tubule connections in series with the distal cysts. The latter explanation seems unlikely since one cannot account for the observation that about one–half of the distal cysts have no detectable β_2m in the fluid when, in fact, proximal cysts afferent to the distal cysts would be expected to discharge high levels of β_2m into distal tubule fluid. One might suppose that distal cysts with detectable β_2m in the fluid are connected to afferent proximal cysts, but this seems unlikely as the highest FD_{β_2m} in distal cysts was 0.19, and the mean FD_{β_2m} of proximal cysts was 0.985. For a proximal to distal cyst ratio of 4:1 [9] in the simplest case of equal flow rates in all cystic and non-cystic segments, one would expect a FD_{β_2m} of 0.25, and the average value is considerably below this. The most economical explanation for the low β_2m levels in distal cysts is to suppose that β_2m is filtered by the glomeruli and that the protein is reabsorbed by proximal tubules. Thus, the levels of creatinine *higher* than in serum [9] and the β_2m levels in distal cyst fluids *lower* than in proximal cyst fluid or serum are consistent with the view that the fluid in distal cysts is derived in part from glomerular filtrate. Recent studies (Grantham, J., unpublished studies) indicate that a significant fraction of the cysts may not be connected to afferent tubule segments. This may account for the finding that some of the distal cysts contain no detectable β_2m, and in some the creatinine level is not higher than in plasma.

Normal proximal tubules reabsorb nearly all of the filtered β_2m [11]. The observation that β_2m levels in proximal cysts are approximately equal to serum values indicates that the abnormal epithelium cannot generate or maintain a gradient for this protein, despite contact between fluid and epithelium much longer than in normal proximal tubules. The study of cyst β_2m levels following renal transplantation indicates that the proximal cyst epithelial permeability to β_2m is relatively low, but despite this the β_2m absorptive mechanism is inadequate to generate or maintain a gradient, whereas distal cysts, which are derived from tubule segments that do not ordinarily reabsorb β_2m, maintain steep gradients for long periods of time. Electron microscopy studies reveal that most proximal cysts do not have apical brush borders, but that the junctional complexes of proximal and distal cysts are identical to those of proximal and distal tubules [7]. As protein and polypeptide absorption in proximal tubules depends on endocytic processes in the apical membranes [11], the absence of brush borders may reflect a more generalized functional abnormality in the apical surface of proximal cyst epithelium. The current data also suggest that the intercellular junctional complexes of proximal cysts are permeable to β_2m, and that distal cyst junctions are impermeable to the protein.

The inability of individual cystic proximal tubules to develop or maintain an transepithelial β_2m gradient is not reflected in the final urine of non-azotemic ADPKD subjects, despite the marked anatomic renal distortion in these patients revealed by computed tomography [2]. In these patients the FE_{β_2m} was indistinguishably different from normal. This finding indicates strongly that the process that causes impaired reabsorption of β_2m in cystic proximal tubules does not affect the non-cystic tubules. In other words, this functional information indicates that cyst formation, at least in non-azotemic patients, is a highly focal process.

Since most proximal cysts with patent efferent tubule connections probably discharge high concentrations of β_2m into the distal tubules, one can infer from the data in Table 1 and Figure 3 that only a small proportion of the proximal tubules in the non-azotemic subjects contain one or more cysts, otherwise the fractional excretion of β_2m in final urine would be much higher than normal. The exact proportion of proximal tubules that are cystic cannot be adduced from the current studies, but it appears that in non-azotemic patients only a few percent of the nephrons contributing to the final urine may contain proximal cysts. As the disease progresses, either more nephrons are recruited to form cysts, or the function of the non-cystic nephrons is compromised to account for the rise in FE_{β_2m} and serum β_2m levels. The level of β_2m in serum is increased in all renal diseases when glomerular filtration rate is decreased [23, 24]. In diseased kidneys, a reduction in the filtered load of β_2m apparently leads to an increased concentration of the protein in serum due to the limited capacity of residual proximal tubules to absorb and degrade it [6]. We cannot differentiate between cystic recruitment of additional nephrons or saturation of the β_2m reabsorption mechanism in end–stage kidneys on the basis of current data, but in view of the observation that ADPKD progresses to renal insufficiency over a relatively rapid course in many adult patients [2, 25] without an accelerated increase in kidney size, it seems more likely that the obliteration of non-cystic nephrons leading to plasma accumulation is a principle factor in the abnormal excretion of β_2m rather than the formation of new cysts.

In summary, the studies of β_2m excretion in urine and accumulation in renal cysts strengthens the view that cysts derive from nephron segments, but that cystic proximal tubule epithelium is unable to maintain concentration gradients of the protein between cyst fluid and plasma. The overall renal handling of β_2m is not unusual in non-azotemic ADPKD patients, suggesting that the marked anatomic distortion of the parenchyma due to the cysts does not alter the tubular processing of β_2m in contiguous normal proximal tubules. Finally, we have interpreted the results to indicate that the massive size of polycystic kidneys is due to the remarkable enlargement of a relatively small number of nephrons.

Acknowledgments

We thank Ralph Butkowski, PhD. and Bo Wisdom for assistance with the HPLC method. Bonnie Danley, Joyce Blair and Alice Algie provided secretarial assistance. This study was supported in part by HHS grants AM 33003 and AM 13476. Dr. Birenboim is recipient of a fellowship award from the Kansas Heart Association.

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